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(54) Title: BETA CHAIN OF THE HUMAN GM-CSF RECEPTOR AND USES THEREOF

(57) Abstract

Nucleic acids encoding the β-chain of the human granulocyte-macrophage colony stimulating factor (GM-CSF) receptor, as well as the β-chain itself, are provided. The β-chain may be expressed with the α-chain in cellular hosts to form compositions useful in screening agonists and antagonists of human GM-CSF.

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+ DESIGNATIONS OF "SU"

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Beta chain of the human GM-CSF receptor and uses thereof

Field of the Invention

The invention relates generally to the human granulocyte-macrophage colony stimulating factor (GM-CSF) receptor, and more particularly, to the synthesis of a human GM-CSF receptor component and to the use of the receptor component for screening agonists and antagonists of human GM-CSF.

BACKGROUND

Circulating blood cells are constantly replaced by newly developed cells. Replacement blood cells are formed in a process termed hematopoiesis which involves the production of at least eight mature blood cell types within two major lineages: (1) the myeloid lineage which includes red blood cells (erythrocytes), macrophages (monocytes), eosinophilic granulocytes, megakaryocytes (platelets), neutrophilic granulocytes, basophilic granulocytes (mast cells); and (2) the lymphoid lineage which includes T lymphocytes and B lymphocytes [Burgess and Nicola, Growth Factors and Stem Cells (Academic Press, New York, 1983)]. Much of the control of blood-cell formation is mediated by a group of interacting glycoproteins termed colony stimulating factors (CSFs). These glycoproteins are so named because of the in vivo and in vitro assays used to detect their presence. Techniques for the clonal culture of hematopoietic cells in semisolid culture medium have been especially important in the development of in vitro assays. In such cultures, individual progenitor cells (i.e., cells developmentally committed to a particular lineage, but still capable of proliferation) are able to proliferate to form a colony of

maturing progeny in a manner which is believed to be essentially identical to the comparable process in vivo. The role of CSFs in hematopoiesis is the subject of many reviews, and is of great interest to clinical investigators who must treat blood diseases or deficiencies; e.g. Metcalf, The Hemopoietic Colony Stimulating Factors (Elsevier, New York, 1984); Clark and Kamen, Science, Vol. 236, pgs. 1229-1237 (1987); Sachs, Science, Vol. 238, pgs. 1374-1379 (1987); Dexter et al., eds., Colony Stimulating Factors (Dekker, New York, 1990); and Morstyn et al., Cancer Investigation, Vol. 7, pgs. 443-456 (1989).

CSFs are believed to play a role in the development and progression of myeloid leukemias; e.g. Metcalf, Hamatol. Bluttransfus., Vol. 31, pgs. 16-25 (1987). Myeloid leukemias are clonal neoplasms of granulocyte-macrophage precursor cells, which fall into two major groups: chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). CML is characterized by expansion in the marrow of the granulocyte-monocyte population at all stages of maturation, with massive enlargement of hematopoietic populations in the spleen and blood. Whereas chemotherapy is successful in reducing the excessive size of the leukemic cell populations, conventional regimens have not succeeded in preventing terminal acute transformation (of progressively higher proportions of cells into immature or abnormal forms) or in extending the life spans of afflicted patients (Metcalf, cited above, 1984). AML is characterized by an accumulation of immature granulocyte-monocyte blast cells with often little or no evidence of maturing granulocyte-monocyte cells. The disease primarily involves the bone marrow, and spleen enlargement usually is only moderate. Total blood nucleated cells may or may not be elevated, but there is a high proportion of immature blast cells associated with relatively few mature cells. There is usually an associated anemia, thrombocytopenia and a relative absence in the marrow and peripheral blood of mature granulocytes and monocytes. Death usually results from uncontrollable hemorrhage or overwhelming infections (Metcalf, cited above, 1984).

It is believed that both forms of leukemia are driven by abnormal production of, or responsiveness to, colony stimulating factors, particularly GM-CSF. In particular, it has been shown that leukemic cells from some AML patients are capable of autonomous proliferation in vitro because they express GM-CSF constitutively, and that such autonomous proliferation can be inhibited by the addition of GM-CSF neutralizing antiserum [Young et al., Blood, Vol. 68, pgs. 1178-1181 (1986)]. It is believed that myeloid leukemias, in particular AML, may be treated by blocking the ability of GM-CSF to stimulate cell growth.

Recently, a low-affinity receptor of human GM-CSF, referred to herein as the α–chain, has been cloned and characterized [Gearing et al., EMBO J., Vol. 8, pgs. 3667-3676 (1989)]. The availability of a high affinity human GM-CSF receptor would provide a valuable tool for screening candidate GM-CSF agonists and antagonists.

SUMMARY OF THE INVENTION

The invention is directed to a component of the human GM-CSF receptor, referred to herein as the β-chain of the human GM-CSF receptor, and to compositions thereof which bind with high affinity to human GM-CSF. The invention includes allelic and genetically engineered variants of the β-chain receptor and nucleic acids encoding the β-chain receptor and its allelic and genetically engineered variants. Preferably, the receptor component of the invention is selected from the group of polypeptides of the open reading frame defined by the amino acid sequence given in SEQ ID NO. 2.

Most preferably, the receptor component of the invention is defined by the amino acid sequence given in SEQ ID NO 2 but lacking the signal sequence.

Although the Formula given in SEQ ID NO. 2, with or without the leader sequence, includes the intracellular domain of the β-chain of the receptor, it is clear that a truncated sequence (with or without the leader sequence) that retains its extracellular and

transmembrane domains and its ability of operably associating with the α–chain falls within the concept of the invention.

The invention is based in part on the discovery and cloning of cDNAs which are capable of expressing proteins that bind to human GM-CSF with high affinity. One such clone, designated pKH97, was deposited with the American Type Culture Collection (ATCC) (Rockville, MD) under accession number 40847 on July 17th 1990. The invention includes nucleic acids (i) that are effectively homologous to the cDNA insert of pKH97, and (ii) that encode proteins that form high affinity GM-CSF receptors in association with the low affinity α-chain receptor protein, e.g. as encoded by pKH125, also deposited with the ATCC under accession number 40848 on July 17th 1990. As used herein, high affinity in reference to GM-CSF receptor binding means that GM-CSF binds to the associated α– and β–chains of the receptor with a binding constant that is at least an order of magnitude less than that for binding to either component alone. More preferably, high affinity means that GM-CSF binds to the associated α– and β–chains of the receptor with a binding constant, K_d, less than 1 nM; and most preferably, less than 200 pM.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates the binding of ^{125}I-labeled human GM-CSF to COS 7 cells transiently co-transfected with KH97 and pKH125.

Figure 1B illustrates the binding of ^{125}I-labeled human GM-CSF to NIH3T3 cells stably transfected with KH97 and pKH125.

Figure 2A illustrates the association rate of ^{125}I-labeled human GM-CSF to the NIH3T3 stable transfectants.

Figure 2B illustrates the dissociation rate of ^{125}I-labeled human GM-CSF to the NIH3T3 stable transfectants.

Figure 3 is a restriction map of the vector pME18.

DETAILED DESCRIPTION OF THE INVENTION

I. Obtaining and Expressing cDNAs for the β-Chain of the Human GM-CSF Receptor

The term "effectively homologous" as used herein means that the nucleotide sequence is capable of being detected by a hybridization probe derived from a cDNA clone of the invention. The exact numerical measure of homology necessary to detect nucleic acids coding for a receptor β-chain depends on several factors including (1) the homology of the probe to non-β-chain coding sequences associated with the target nucleic acids, (2) the stringency of the hybridization conditions, (3) whether single stranded or double stranded probes are employed, (4) whether RNA or DNA probes are employed, (5) the measures taken to reduce nonspecific binding of the probe, (6) the nature of the method used to label the probe, (7) the fraction of guanidine and cytosine bases in the probe, (8) the distribution of mismatches between probe and target, (9) the size of the probe, and the like. Preferably, an effectively homologous nucleic acid sequence is at least seventy percent (70%) homologous to the cDNA of the invention. More preferably, an effectively homologous nucleic acid is at least ninety percent (90%) homologous to the cDNA of the invention. Most particularly, an effectively homologous nucleic acid sequence is one whose cDNA can be isolated by a probe based on the nucleic acid sequence of SEQ ID NO. 1 using a standard hybridization protocol with no more than a few false positive signals, e.g. fewer than a hundred. There is an extensive literature that provides guidance in selecting conditions for such hybridizations: e.g., Hames et al., Nucleic Acid Hybridization: A Practical Approach (IRL Press, Washington, D.C., 1985); Gray et al., Proc. Natl. Acad. Sci., Vol. 80, pgs. 5842-5846 (1983); Kafatos et al., Nucleic Acids Research, Vol. 7, pgs. 1541-1552 (1979); and Williams, Genetic Engineering, Vol. 1, pgs. 1-59 (1981), to name a few. By way of example, the nucleic acid of SEQ ID NO. 1 can be used as a probe in colony hybridization assays as described by Benton and Davis, Science, Vol. 196, pg. 180 (1977). Preferably, low stringency conditions are employed for

the probe employed. (The dissociation temperature depends on the probe length.) For example, for a probe of about 20-40 bases a typical prehybridization, hybridization, and wash protocol is as follows: (1) prehybridization: incubate nitrocellulose filters containing the denatured target DNA for 3-4 hours at 55°C in 5x Denhardt's solution, 5x SSPE (20x SSPE consists of 174 g NaCl, 27.6 g $NaH_2PO_4 \cdot H_2O$, and 7.4 g EDTA in 800 ml H_2O adjusted to pH 7.4 with 10 N NaOH), 0.1% SDS, and 100 µg/ml denatured salmon sperm DNA, (2) hybridization: incubate filters in prehybridization solution plus probe at 55°C for 2 hours, (3) wash: three 15 minute washes in 300-500 ml volumes of 6x SSC and 0.1% SDS at room temperature, followed by a final 1-1.5 minute wash in 300-500 ml of 1x SSC and 0.1% SDS at 55°C. Other equivalent procedures, e.g. employing organic solvents such as formamide, are well known in the art.

Homology as the term is used herein is a measure of similarity between two nucleotide (or amino acid) sequences. Homology is expressed as the fraction or percentage of matching bases (or amino acids) after two sequences (possibly of unequal length) have been aligned. The term alignment is used in the sense defined by Sankoff and Kruskal in chapter one of Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison (Addison-Wesley, Reading, MA, 1983). Roughly, two sequences are aligned by maximizing the number of matching bases (or amino acids) between the two sequences with the insertion of a minimal number of "blank" or "null" bases into either sequence to bring about the maximum overlap. Given two sequences, algorithms are available for computing their homology: e.g., Needleham and Wunsch, J. Mol. Biol., Vol. 48, pgs. 443-453 (1970); and Sankoff and Kruskal (cited above), pgs. 23-29. Also, commercial services and software packages are available for performing such comparisons, e.g. Intelligenetics, Inc. (Mountain View, CA), and University of Wisconsin Genetics Computer Group (Madison, Wisconsin).

Probes based on the nucleic acid sequence of SEQ ID NO. 3 can be synthesized on commercially available DNA synthesizers,

e.g. Applied Biosystems model 381A, using standard techniques, e.g. Gait, Oligonucleotide Synthesis: A Practical Approach, (IRL Press, Washington D.C., 1984). It is preferable that the probe be at least 18-30 bases long. More preferably, the probe is at least 100-200 bases long. Probes of the invention can be labeled in a variety of ways standard in the art, e.g. with radioactive labels [Berent et al., Biotechniques, pgs. 208-220 (May/June 1985); Meinkoth et al., Anal. Biochem., Vol. 138, pgs. 267-284 (1984); Szostak et al., Meth. Enzymol., Vol. 68, pgs. 419-429 (1979); and the like], or with non-radioactive labels [Chu et al., DNA, Vol. 4, pgs. 327-331 (1985); Jablonski et al., Nucleic Acids Research, Vol. 14, pgs. 6115-6128 (1986); and the like].

Hybridization probes can also be used to screen candidate sources of β-chain mRNA prior to library construction, e.g. by RNA blotting: Maniatis et al., Molecular Cloning: A Laboratory Manual, pgs. 202-203 (Cold Spring Harbor Laboratory, N.Y., 1982); or Hames and Higgins, eds., pgs. 139-143 in Nucleic Acids Hybridization (IRL Press, Washington, D.C., 1985). Sources of mRNA encoding the desired polypeptides include cell populations or cell lines that express, or can be induced to express, large numbers of GM-CSF receptors on their surfaces, e.g. in excess of 3000-5000.

Preferably, the α- and β-chains of the GM-CSF receptor are co-transfected into a mammalian expression system (i.e. host-expression vector combination). Many reviews are available which provide guidance for making choices and/or modifications of specific mammalian expression systems: e.g. (to name a few), Kucherlapati et al., Critical Reviews in Biochemistry, Vol. 16, Issue 4, pgs. 349-379 (1984), and Banerji et al., Genetic Engineering, Vol. 5, pgs. 19-31 (1983), review methods for transfecting and transforming mammalian cells; Reznikoff and Gold, eds., Maximizing Gene Expression (Butterworths, Boston, 1986) review selected topics in gene expression in E. coli, yeast, and mammalian cells; and Thilly, Mammalian Cell Technology (Butterworths, Boston, 1986) reviews mammalian expression systems. Likewise, many reviews are available which describe techniques and conditions for linking and/or manipulating specific cDNAs and

expression control sequences to create and/or modify expression vectors suitable for use with the present invention; e.g. Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, N.Y., 1982); Glover, DNA Cloning: A Practical Approach, Vol. I and II (IRL Press, Oxford, 1985), and Perbal, A Practical Guide to Molecular Cloning (John Wiley & Sons, N.Y., 1984), to name only a few.

Several DNA tumor viruses have been used as vectors for mammalian hosts. Particularly important are the numerous vectors which comprise SV40 replication, transcription, and/or translation control sequences coupled to bacterial replication control sequences; e.g., the pcD vectors developed by Okayama and Berg, disclosed in Mol. Cell. Biol., Vol. 2, pgs. 161-170 (1982) and Mol. Cell. Biol., Vol. 3, pgs. 280-289 (1983), both of which are incorporated herein by reference; the SV40 vectors disclosed by Hamer in Genetic Engineering, Vol. 2, pgs. 83-100 (1980), and U.S. Patent 4,599,308, both of which are incorporated herein by reference; and the vectors additionally containing adenovirus regulatory elements, disclosed by Kaufman and Sharp, in Mol. Cell. Biol., Vol. 2, pgs. 1304-1319 (1982), and by Clark et al. in U.S. patent 4,675,285, both of which are incorporated herein by reference. COS7 monkey cells, described by Gluzman, Cell, Vol. 23, pgs. 175-182 (1981) and available from the ATCC (accession no. CRL 1651), are usually the preferred hosts for the above vectors. SV40-based vectors suitable for mammalian receptor expression have been developed by Aruffo and Seed [Proc. Natl. Acad. Sci., Vol. 84, pgs. 3365-3369 and 8573-8577 (1987)].

II. Binding Assays

Binding assays are accomplished by letting a ligand of unknown specificity or affinity compete with a known amount or concentration of labeled human GM-CSF for receptor binding sites of a sample of cells transfected or transformed with pKH97 and pKH125, or their equivalents. Preferably, the GM-CSF is labeled by radioiodination using standard protocols, e.g. reaction with 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril described by Fraker et al.,

Biochem Biophys. Res. Commun., Vol. 80, pgs. 849-857 (1978) (and available from Pierce Chemical Co. as Iodogen). Generally, the binding assay is conducted as described by Lowenthal et al., J. Immunol., Vol 140, pgs. 456-464 (1988), which is incorporated by reference. Briefly, aliquots of cells are incubated in the presence of ^{125}I-labeled human GM-CSF in a final volume of 200 µl culture medium in microfuge tubes at 4°C. Cell-bound ^{125}I-labeled GM-CSF was separated from non-bound ^{125}I-labeled GM-CSF by centrifugation through an oil gradient (10,000 x G for 2 min). Nonspecific binding is measured in the presence of a 100-fold excess of partially purified unlabeled human GM-CSF.

The following Examples serve to illustrate the invention but do not limit it in any way:

EXAMPLES

Example I. Construction of cDNA library from TF-1 cells and isolation of pKH97 and pKH125

Poly(A)$^+$ RNA isolated from TF-1 cells (Kitamura et al., J. Cell. Physiol., Vol. 140, pgs. 323-334 (1989)) by the guanidium isothiocyanate method (Chirgwin et al., Biochemistry, Vol. 18, pgs. 5294-5299 (1978)) was converted to double-stranded cDNA using oligo-(dT) primers. After BstXI linkers were ligated to both ends of the cDNAs, the cDNAs were digested with XbaI (the 3'-region fortuitously containing a unique XbaI site) and re-cloned into BstXI/XbaI-digested pME18, an SV40-based mammalian expression vector (see Figure 3). pKH97 was isolated by using probes constructed from initially isolated truncated cDNAs. The truncated cDNAs were isolated using a ^{32}P-labeled mouse interleukin-3 receptor cDNA fragment (described by Itoh et al., Science, Vol. 247, pgs. 324-334 (1990)) as a hybridization probe under low stringency conditions (hybridization at 42°C with 6xSSPE in the presence of 20% formamide and washing at 50°C with 2xSSPE). pKH97 was transfected into COS 7 cells by a standard protocol, e.g. as described by Yokota et al., Proc. Natl. Acad. Sci., Vol. 84, pgs. 7388-7392 (1987) (5 µg of plasmid DNA were transfected into

semi-confluent COS 7 cells by the DEAE-dextran method; 72 hours after transfection, the cells were harvested for binding assays, using iodinated cytokines as described below). No specific binding was displayed by any of the following human cytokines at the following concentrations: IL-2 (1 nM), IL-3 (20 nM), IL-4 (1 nM), IL-5 (5 nM), GM-CSF (20 nM), and EPO (10 nM).

A cDNA encoding the α-chain of the human GM-CSF receptor was isolated from the same library using the polymerase chain reaction with specific oligonucleotide primers corresponding to the 5'-untranslated and the 3'-untranslated regions of the cDNA described by Gearing et al., EMBO J., Vol. 8, pgs. 3667-3676 (1989). Inserting the isolated cDNA into pME18 gave pKH125.

Example II. Co-transfection of pKH97 and pKH125 into COS 7 cells

A total of 5 μg of equal amounts of pKH97 and pKH125 plasmid DNA was transfected into semi-confluent COS 7 cells by the DEAE-dextran method. 72 hours after transfection, the cells were harvested and analyzed in GM-CSF binding assays. Duplicates of 2×10^5 COS 7 cells in 0.1 ml of RPMI 1640 containing 10% fetal calf serum, 2 mM EDTA, 0.02% sodium azide and 20 mM Hepes (pH 7.4) were incubated for 3 hours at 4°C with various concentrations of ^{125}I-labeled human GM-CSF with or without an excess amount of non-labeled human GM-CSF. The cell-bound radioactivity was measured by separating the cells from free ligand by centrifugation through an oil layer, as described by Schreurs et al., Growth Factors, Vol. 2, pgs. 221-233 (1990). GM-CSF was iodinated by a standard protocol [Chiba et al., Leukemia, Vol. 4, pgs. 22-36 (1990)]. Briefly, 5 μg of *E. coli*-produced human GM-CSF was incubated in 30-50 μl of 50 mM sodium borate buffer (pH 8.0) with 1 mCi of the dried Bolton-Hunter reagent for 12-16 hours at 4°C. Glycine was added to 2.5 mg/ml to stop the reaction and the iodinated GM-CSF was separated from the free Bolton-Hunter reagent by a PD-10 column. The iodinated human GM-CSF had a specific radioactivity of $(4\text{-}8) \times 10^7$ cpm/μg and was stable for about two months in Hepes-

buffered Hank's balanced salt solution containing 0.1% gelatin, 0.1% bovine serum albumin, and 0.02% sodium azide.

Figure 1A shows the receptor binding data. Open circles correspond to COS 7 cells (controls) transfected with pKH125 and pME18 (same vector as pKH97, but without the cDNA insert). Closed circles correspond to COS 7 cells transfected with pKH125 and pKH97. The Scatchard plots of the binding data are shown. The inserted graphs show equilibrium binding profiles. As can be seen from the data, both high (K_d=120 pM) and low (K_d=6.6 nM) affinity binding sites are indicated (the K_d values being computed by the LIGAND program, De Lean et al., Mol. Pharmacol., Vol. 21, pgs. 5-16 (1982)).

Example III. Co-transfection of pKH97 and pKH125 into NIH3T3 Cells

A DNA fragment containing the neomycin-resistance gene, neo, was inserted into pKH97 to form pKH97neo. NIH3T3 cells were stably transfected with pKH97neo and pKH125 by the calcium-phosphate procedure, described by Chen and Okayama, Mol. Cell. Biol., Vol. 7, pgs. 2745-2752 (1987), which reference is incorporated by reference. Stable transfectants were selected by 1 mg/ml of G418. Figure 1B shows the binding data for the transfected NIH3T3 cells. The open circles correspond to control NIH3T3 cells transfected with pKH97neo and pME18. Closed circles correspond to NIH3T3 cells transfected with pKH97neo and pKH125. The latter displayed high affinity binding with a K_d of 170 pM. Labeled GM-CSF association and dissociation rates were also examined in the transfected NIH3T3 cells. Figures 2A and 2B illustrate the data. Open circles correspond to NIH3T3 cells expressing only the α-chain. Closed circles correspond to NIH3T3 cells expressing both the α-chain and β-chain of the GM-CSF receptor. The latter displayed a much slower rate of dissociation ($T_{1/2}$=2 min versus $T_{1/2}$=360 min).

Example IV. Use of Co-transfected COS 7 cells to screen for GM-CSF Antagonists

Aliquots of COS 7 cells co-transfected with pKH97 and pKH125 as described above are distributed to wells of microtiter

plates in 200 µl of medium containing ^{125}I-labeled human GM-CSF at concentrations of 100 pM, 500 pM, and 1 nM. 100 µl samples of microbial supernatants free of cells are added to the transfected COS 7 cells at each of the different concentrations of ^{125}I-labeled GM-CSF. After incubating for 3 hours the COS 7 cells are harvested and assayed for bound radioactivity. COS 7 cells with low counts of bound radioactivity correspond to microbial samples containing candidate antagonists or agonists of human GM-CSF.

The 'stuffer' region of the vector pME18 is described by Seed et al., Proc. Natl. Acad. Sci., Vol. 84 (1987), pp. 3365-3369.

On July 17th 1990, Applicants deposited pKH97 and pKH125 with the American Type Culture Collection, Rockville, MD, USA (ATCC), under accession numbers 40847 and 40848, respectively. These deposits were made for international purposes under the Budapest Treaty, and also for US purposes under conditions as provided under the ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposits will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 USC 122 and 37 CFR 1.14, and will be made available to the public upon issue of a U.S. patent, which requires that the deposit be maintained. Availability of the deposited plasmids is not to be construed as a license to practise the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments were chosen and described in order to best explain the principles of the invention to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

SEQUENCE LISTING

SEQ ID NO: 1

SEQUENCE TYPE: DNA Sequence

SEQUENCE LENGTH: 3475 bases

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: DNA molecule

ORIGINAL SOURCE ORGANISM: Human

PROPERTIES: DNA sequence encoding Human GM-SCF receptor

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GAAGACTGGT CTCTCCCACC ACACAGAGGC CTGGAGGAGG CAGAGGCCAG GAGGGAGAGG   60
TCCCAAGAGC CTGTGAAATG GGTCTGGCCT GGCTCCCAGC TGGGCAGGAA CACAGGACTT  120
CAGGACACTA AGGACCCTGT CATGCCCATG GCCAGCACCC ACCAGTGCTG GTGCCTGCCT  180
GTCCAGAGCT GACCAGGGAG ATG GTG CTG GCC CAG GGG CTG CTC TCC ATG GCC  233
CTG CTG GCC CTG TGC TGG GAG CGC AGC CTG GCA GGG GCA GAA GAA ACC  281
ATC CCG CTG CAG ACC CTG CGC TGC TAC AAC GAC TAC ACC AGC CAC ATC  329
ACC TGC AGG TGG GCA GAC ACC CAG GAT GCC CAG CGG CTC GTC AAC GTG  377
ACC CTC ATT CGC CGG GTG AAT GAG GAC CTC CTG GAG CCA GTG TCC TGT  425
GAC CTC AGT GAT GAC ATG CCC TGG TCA GCC TGC CCC CAT CCC CGC TGC  473
GTG CCC AGG AGA TGT GTC ATT CCC TGC CAG AGT TTT GTC GTC ACT GAC  521
GTT GAC TAC TTC TCA TTC CAA CCA GAC AGG CCT CTG GGC ACC CGG CTC  569
ACC GTC ACT CTG ACC CAG CAT GTC CAG CCT CCT GAG CCC AGG GAC CTG  617
CAG ATC AGC ACC GAC CAG GAC CAC TTC CTG CTG ACC TGG AGT GTG GCC  665
CTT GGG AGT CCC CAG AGC CAC TGG TTG TCC CCA GGG GAT CTG GAG TTT  713
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CTC CTC TCC AAC ACC TCC CAG GCC ACC CTG GGG CCA GAG CAC CTC ATG  809
CCC AGC AGC ACC TAC GTG GCC CGA GTA CGG ACC CGC CTG GCC CCA GGT  857
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GAC TCC CAG CCA GGG GAT GAG GCC CAG CCC CAG AAC CTG GAG TGC TTC  953
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GTG GCC AGC TCG GTC TCC TTT GGC CTA TTC TAC AAG CCC AGC CCA GAT 1049
GCA GGG GAG GAA GAG TGC TCC CCA GTG CTG AGG GAG GGG CTC GGC AGC 1097
CTC CAC ACC AGG CAC CAC TGC CAG ATT CCC GTG CCC GAC CCC GCG ACC 1145
CAC GGC CAA TAC ATC GTC TCT GTT CAG CCA AGG AGG GCA GAG AAA CAC 1193
ATA AAG AGC TCA GTG AAC ATC CAG ATG GCC CCT CCA TCC CTC AAC GTG 1241
ACC AAG GAT GGA GAC AGC TAC AGC CTG CGC TGG GAA ACA ATG AAA ATG 1289
CGA TAC GAA CAC ATA GAC CAC ACA TTT GAG ATC CAG TAC AGG AAA GAC 1347
ACG GCC ACG TGG AAG GAC AGC AAG ACC GAG ACC CTC CAG AAC GCC CAC 1395
AGC ATG GCC CTG CCA GCC CTG GAG CCC TCC ACC AGG TAC TGG GCC AGG 1443
GTG AGG GTC AGG ACC TCC CGC ACC GGC TAC AAC GGG ATC TGG AGC GAG 1491
TGG AGT GAG GCG CGC TCC TGG GAC ACC GAG TCG GTG CTG CCT ATG TGG 1539
GTG CTG GCC CTC ATC GTG ATC TTC CTC ACC ACT GCT GTG CTC CTG GCC 1587
CTC CGC TTC TGT GGC ATC TAC GGG TAC AGG CTG CGC AGA AAG TGG GAG 1645
GAG AAG ATC CCC AAC CCC AGC AAG AGC CAC CTG TTC CAG AAC GGG AGC 1683
GCA GAG CTT TGG CCC CCA GGC AGC ATG TCG GCC TTC ACT AGC GGG AGT 1721
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CCC CCA CAC CAG GGG CCG TGG GGC AGC CGC TTC CCT GAG CTG GAG GGG 1769
GTG TTC CCT GTA GGA TTC GGG GAC AGC GAG GTG TCA CCT CTC ACC ATA 1817
GAG GAC CCC AAG CAT GTC TGT GAT CCA CCA TCT GGG CCT GAC ACG ACT 1865
CCA GCT GCC TCA GAT CTA CCC ACA GAG CAG CCC CCC AGC CCC CAG CCA 1913
GGC CCG CCT GCC GCC TCC CAC ACA CCT GAG AAA CAG GCT TCC AGC TTT 1961
GAC TTC AAT GGG CCC TAC CTG GGG CCG CCC CAC AGC CGC TCC CTA CCT 2009
GAC ATC CTG GGC CAG CCG GAG CCC CCA CAG GAG GGT GGG AGC CAG AAG 2057
TCC CCA CCT CCA GGG TCC CTG GAG TAC CTG TGT CTG CCT GCT GGG GGG 2105
CAG GTG CAA CTG GTC CCT CTG GCC CAG GCG ATG GGA CCG GGA CAG GCC 2153
GTG GAA GTG GAG AGA AGG CCG AGC CAG GGG GCT GCA GGG AGT CCC TCC 2201
CTG GAG TCC GGG GGA GGC CCT GCC CCT CCT GCT CTT GGG CCA AGG GTG 2249
GGA GGA CAG GAC CAA AAG GAC AGC CCT GTG GCT ATA CCC ATG AGC TCT 2297
GGG GAC ACT GAG GAC CCT GGA GTG GCC TCT GGT TAT GTC TCC TCT GCA 2345
GAC CTG GTA TTC ACC CCA AAC TCA GGG GCC TCG TCT GTC TCC CTA GTT 2393
CCC TCT CTG GGC CTC CCC TCA GAC CAG ACC CCC AGC TTA TGT CCT GGG 2441
CTG GCC AGT GGA CCC CCT GGA GCC CCA GGC CCT GTG AAG TCA GGG TTT 2489
GAG GGC TAT GTG GAG CTC CCT CCA ATT GAG GGC CGG TCC CCC AGG TCA 2537
CCA AGG AAC AAT CCT GTC CCC CCT GAG GCC AAA AGC CCT GTC CTG AAC 2585
CCA GGG GAA CGC CCG GCA GAT GTG TCC CCA ACA TCC CCA CAG CCC GAG 2633
GGC CTC CTT GTC CTG CAG CAA GTG GGC GAC TAT TGC TTC CTC CCC GGC 2681
CTG GGG CCC GGC CCT CTC TCG CTC CGG AGT AAA CCT TCT TCC CCG GGA 2729
CCC GGT CCT GAG ATC AAG AAC CTA GAC CAG GCT TTT CAA GTC AAG AAG 2777
CCC CCA GGC CAG GCT GTG CCC CAG GTG CCC GTC ATT CAG CTC TTC AAA 2825
GCC CTG AAG CAG CAG GAC TAC CTG TCT CTG CCC CCT TGG GAG GTC AAC 2873
AAG CCT GGG GAG GTG TGT TGA GACC CCCAGGCCTA GACAGGCAAG GGGATGGAGA 2928
GGGCTTGCCT TCCCTCCCGC CTGACCTTCC TCAGTCATTT CTGCAAAGCC AAGGGGCAGC 2988
CTCCTGTCAA GGTAGCTAGA GGCCTGGGAA AGGAGATAGC CTTGCTCCGG CCCCCTTGAC 3048
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ACACATTTTT CCTGTCAGGT TAACTTATTT GTAGGTTCTG CATTATTAGA ACTTTCTAGA 3168
TATACTCATT CCATCTCCCC CTCATTTTTT TAATCAGGTT TCCTTGCTTT TGCCATTTTT 3228
CTTCCTTCTT TTTTCACTGA TTTATTATGA GAGTGGGGCT GAGGTCTGAG CTGAGCCTTA 3288
TCAGACTGAG ATGCGGCTGG TTGTGTTGAG GACTTGTGTG GGCTGCCTGT CCCCGGCAGT 3348
CGCTGATGCA CATGACATGA TTCTCATCTG GGTGCAGAGG TGGGAGGCAC CAGGTGGGCA 3408
CCCGTGGGGG TTAGGGCTTG GAAGAGTGGC ACAGGACTGG GCACGCTCAG TGAGGCTCAG 3468
GGAATTC 3475

SEQ ID NO: 2

SEQUENCE TYPE: Amino acid sequence

SEQUENCE LENGTH: 897 amino acid residues

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: Protein/polypeptide

ORIGINAL SOURCE ORGANISM: Human

PROPERTIES: Human GM-CSF receptor

FEATURES:

Encoded by DNA sequence of SEQ ID NO. 1;
SIGNAL SEQUENCE: -17 to -1;
TRANSMEMBRANE DOMAIN: 422 to 448
POTENTIAL N-LINKED GLYCOSYLATION SITES IN THE
EXTRACELLULAR DOMAIN: 41-43; 174-176; 329-331

```
Met Val Leu Ala Gln Gly Leu Leu Ser Met Ala Leu Leu Ala Leu
        -15                 -10                 -5
Cys Trp Glu Arg Ser Leu Ala Gly Ala Glu Glu Thr Ile Pro Leu
          1               5                  10
Gln Thr Leu Arg Cys Tyr Asn Asp Tyr Thr Ser His Ile Thr Cys
     15                  20                  25
Arg Trp Ala Asp Thr Gln Asp Ala Gln Arg Leu Val Asn Val Thr
     30                  35                  40
Leu Ile Arg Arg Val Asn Glu Asp Leu Leu Glu Pro Val Ser Cys
     45                  50                  55
Asp Leu Ser Asp Asp Met Pro Trp Ser Ala Cys Pro His Pro Arg
     60                  65                  70
Cys Val Pro Arg Arg Cys Val Ile Pro Cys Gln Ser Phe Val Val
     75                  80                  85
Thr Asp Val Asp Tyr Phe Ser Phe Gln Pro Asp Arg Pro Leu Gly
     90                  95                 100
Thr Arg Leu Thr Val Thr Leu Thr Gln His Val Gln Pro Pro Glu
    105                 110                 115
Pro Arg Asp Leu Gln Ile Ser Thr Asp Gln Asp His Phe Leu Leu
    120                 125                 130
Thr Trp Ser Val Ala Leu Gly Ser Pro Gln Ser His Trp Leu Ser
    135                 140                 145
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Pro Gly Asp Leu Glu Phe Glu Val Val Tyr Lys Arg Leu Gln Asp
150 155 160

Ser Trp Glu Asp Ala Ala Ile Leu Leu Ser Asn Thr Ser Gln Ala
165 170 175

Thr Leu Gly Pro Glu His Leu Met Pro Ser Ser Thr Tyr Val Ala
180 185 190

Arg Val Arg Thr Arg Leu Ala Pro Gly Ser Arg Leu Ser Gly Arg
195 200 205

Pro Ser Lys Trp Ser Pro Glu Val Cys Trp Asp Ser Gln Pro Gly
210 215 220

Asp Glu Ala Gln Pro Gln Asn Leu Glu Cys Phe Phe Asp Gly Ala
225 230 235

Ala Val Leu Ser Cys Ser Trp Glu Val Arg Lys Glu Val Ala Ser
240 245 250

Ser Val Ser Phe Gly Leu Phe Tyr Lys Pro Ser Pro Asp Ala Gly
255 260 265

Glu Glu Glu Cys Ser Pro Val Leu Arg Glu Gly Leu Gly Ser Leu
270 275 280

His Thr Arg His His Cys Gln Ile Pro Val Pro Asp Pro Ala Thr
285 290 295

His Gly Gln Tyr Ile Val Ser Val Gln Pro Arg Arg Ala Glu Lys
300 305 310

His Ile Lys Ser Ser Val Asn Ile Gln Met Ala Pro Pro Ser Leu
315 320 325

Asn Val Thr Lys Asp Gly Asp Ser Tyr Ser Leu Arg Trp Glu Thr
330 335 340

Met Lys Met Arg Tyr Glu His Ile Asp His Thr Phe Glu Ile Gln
345 350 355

Tyr Arg Lys Asp Thr Ala Thr Trp Lys Asp Ser Lys Thr Glu Thr
360 365 370

Leu Gln Asn Ala His Ser Met Ala Leu Pro Ala Leu Glu Pro Ser
375 380 385

Thr Arg Tyr Trp Ala Arg Val Arg Val Arg Thr Ser Arg Thr Gly
390 395 400

Tyr Asn Gly Ile Trp Ser Glu Trp Ser Glu Ala Arg Ser Trp Asp
405 410 415

Thr Glu Ser Val Leu Pro Met Trp Val Leu Ala Leu Ile Val Ile
420 425 430

Phe Leu Thr Thr Ala Val Leu Leu Ala Leu Arg Phe Cys Gly Ile
435 440 445

Tyr Gly Tyr Arg Leu Arg Arg Lys Trp Glu Glu Lys Ile Pro Asn
450 455 460

Pro Ser Lys Ser His Leu Phe Gln Asn Gly Ser Ala Glu Leu Trp
465 470 475

Pro Pro Gly Ser Met Ser Ala Phe Thr Ser Gly Ser Pro Pro His
480 485 490

Gln Gly Pro Trp Gly Ser Arg Phe Pro Glu Leu Glu Gly Val Phe
495 500 505

Pro Val Gly Phe Gly Asp Ser Glu Val Ser Pro Leu Thr Ile Glu
510 515 520

Asp Pro Lys His Val Cys Asp Pro Pro Ser Gly Pro Asp Thr Thr
525 530 535

Pro Ala Ala Ser Asp Leu Pro Thr Glu Gln Pro Pro Ser Pro Gln
540 545 550

Pro Gly Pro Pro Ala Ala Ser His Thr Pro Glu Lys Gln Ala Ser
555 560 565

Ser Phe Asp Phe Asn Gly Pro Tyr Leu Gly Pro Pro His Ser Arg
570 575 580

Ser Leu Pro Asp Ile Leu Gly Gln Pro Glu Pro Pro Gln Glu Gly
585 590 595

Gly Ser Gln Lys Ser Pro Pro Pro Gly Ser Leu Glu Tyr Leu Cys
600 605 610

Leu Pro Ala Gly Gly Gln Val Gln Leu Val Pro Leu Ala Gln Ala
615 620 625

Met Gly Pro Gly Gln Ala Val Glu Val Glu Arg Arg Pro Ser Gln
630 635 640

Gly Ala Ala Gly Ser Pro Ser Leu Glu Ser Gly Gly Gly Pro Ala
645 650 655

Pro Pro Ala Leu Gly Pro Arg Val Gly Gly Gln Asp Gln Lys Asp
660 665 670

Ser Pro Val Ala Ile Pro Met Ser Ser Gly Asp Thr Glu Asp Pro
675 680 685

Gly Val Ala Ser Gly Tyr Val Ser Ser Ala Asp Leu Val Phe Thr
690 695 700

Pro Asn Ser Gly Ala Ser Ser Val Ser Leu Val Pro Ser Leu Gly
705 710 715

Leu Pro Ser Asp Gln Thr Pro Ser Leu Cys Pro Gly Leu Ala Ser
720 725 730

Gly Pro Pro Gly Ala Pro Gly Pro Val Lys Ser Gly Phe Glu Gly
735 740 745

Tyr Val Glu Leu Pro Pro Ile Glu Gly Arg Ser Pro Arg Ser Pro
750 755 760

Arg Asn Asn Pro Val Pro Pro Glu Ala Lys Ser Pro Val Leu Asn
765 770 775

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Pro Gly Glu Arg Pro Ala Asp Val Ser Pro Thr Ser Pro Gln Pro
    780                 785                 790

Glu Gly Leu Leu Val Leu Gln Gln Val Gly Asp Tyr Cys Phe Leu
    795                 800                 805

Pro Gly Leu Gly Pro Gly Pro Leu Ser Leu Arg Ser Lys Pro Ser
    810                 815                 820

Ser Pro Gly Pro Gly Pro Glu Ile Lys Asn Leu Asp Gln Ala Phe
    825                 830                 835

Gln Val Lys Lys Pro Pro Gly Gln Ala Val Pro Gln Val Pro Val
    840                 845                 850

Ile Gln Leu Phe Lys Ala Leu Lys Gln Gln Asp Tyr Leu Ser Leu
    855                 860                 865

Pro Pro Trp Glu Val Asn Lys Pro Gly Glu Val Cys
    870                 875                 880
```

CLAIMS:

1. A β-chain of a human granulocyte-macrophage colony stimulating factor receptor substantially free of human non-receptor proteins.

2. The protein of claim 1 wherein said β-chain is a polypeptide defined by the amino acid sequence given in SEQ ID NO. 2.

3. The protein of claim 1 wherein said β-chain is a polypeptide defined by the amino acid sequence given for amino acid residues 1 to 880 in SEQ ID NO. 2.

4. A nucleic acid capable of encoding a polypeptide defined by the amino acid sequence given in SEQ ID NO. 2.

5. The nucleic acid of claim 4 wherein said polypeptide is defined by the amino acid sequence given for amino acid residues 1 to 880 in SEQ ID NO. 2.

6. A nucleic acid which is effectively homologous to the nucleotide sequence of SEQ ID NO. 1 and which encodes a polypeptide capable of forming a high affinity receptor for human granulocyte-macrophage colony stimulating factor, the polypeptide forming the high affinity receptor in operable association with an α-chain of a human granulocyte-macrophage colony stimulating factor.

7. The nucleic acid of claim 6 wherein said high affinity receptor has a binding constant (K_d) with human granulocyte-macrophage colony stimulating factor of less than 1 nM.

8. The nucleic acid of claim 7 wherein said polypeptide is operably associated with said α-chain in a mammalian expression host co-transfected with the nucleic acid and a vector carrying a gene for said α-chain.

9. A method of detecting an antagonist or agonist of human granulocyte-macrophage colony stimulating factor, the method comprising the steps of:

providing a cellular host expressing genes for the α– and β-chains of a human granulocyte-macrophage colony stimulating factor receptor so that the α– and β-chains are operably associated in the membrane of the cellular host;

exposing the cellular host to a known concentration of human granulocyte-macrophage colony stimulating factor and a sample suspected of containing an antagonist or agonist of human granulocyte-macrophage colony stimulating factor, the human granulocyte-macrophage colony stimulating factor being labeled;

removing the cellular host from the sample and the labeled human granulocyte-macrophage colony stimulating factor; and

determining the amount of labeled human granulocyte-macrophage colony stimulating factor that bound to the cellular host.

10. The method of claim 9 wherein said cellular host is a mammalian cell stably transformed with a first vector carrying a gene for said α–chain and a second vector carrying a gene for said β-chain.

11. The method of claim 10 wherein said first vector is pKH125 and said second vector is pKH97.

12. A composition of matter comprising an α-chain and a β-chain of a human granulocyte-macrophage colony stimulating factor receptor, the α-chain and β-chain being in operable association in a non-human cellular host.

13. The composition of matter of claim 12 wherein said α–chain is encoded by the cDNA insert of pKH125 and said β–chain is encoded by the cDNA insert of pKH97.

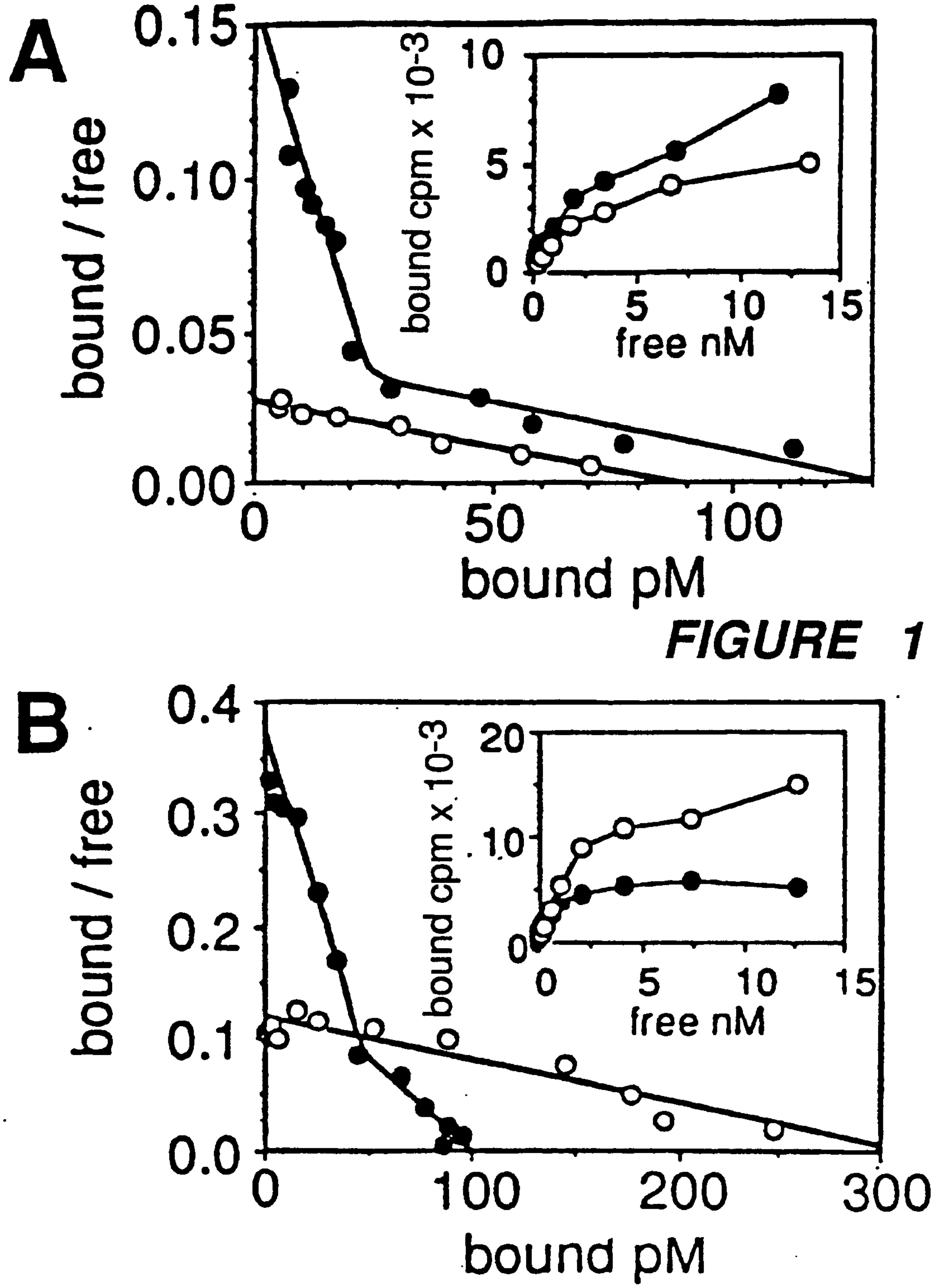

A
0.15
0.10
0.05
0.00
bound / free
0
50
100
bound pM
10
5
0
bound cpm x 10-3
0
5
10
15
free nM
B
0.4
0.3
0.2
0.1
0.0
bound / free
0
100
200
300
bound pM
20
10
0
bound cpm x 10-3
0
5
10
15
free nM

FIGURE 1

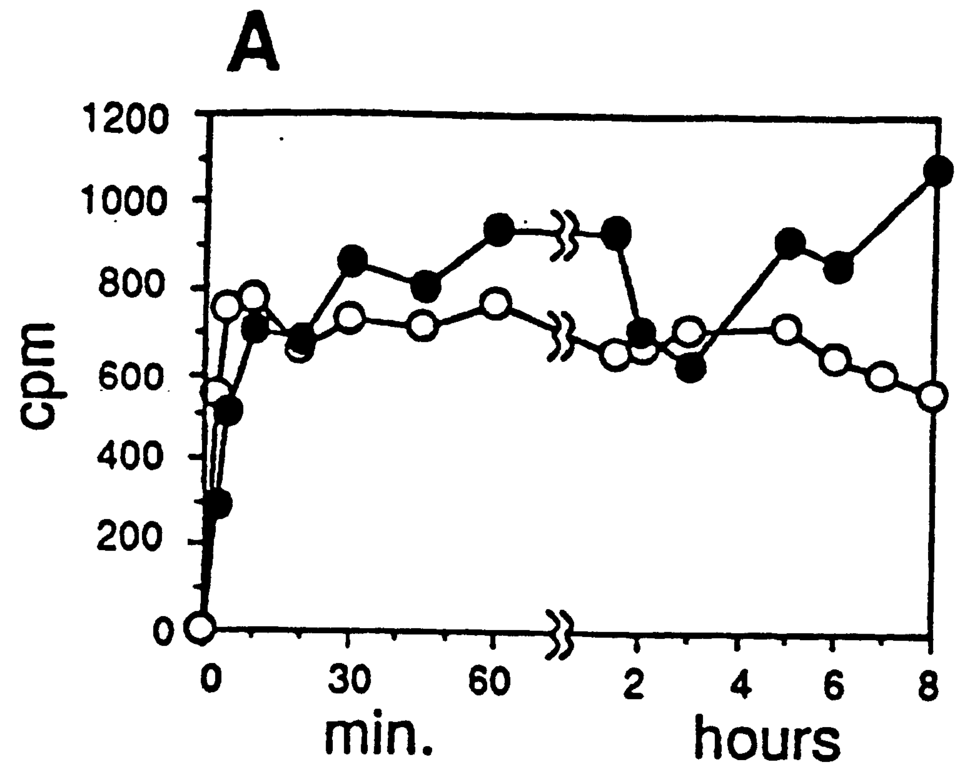
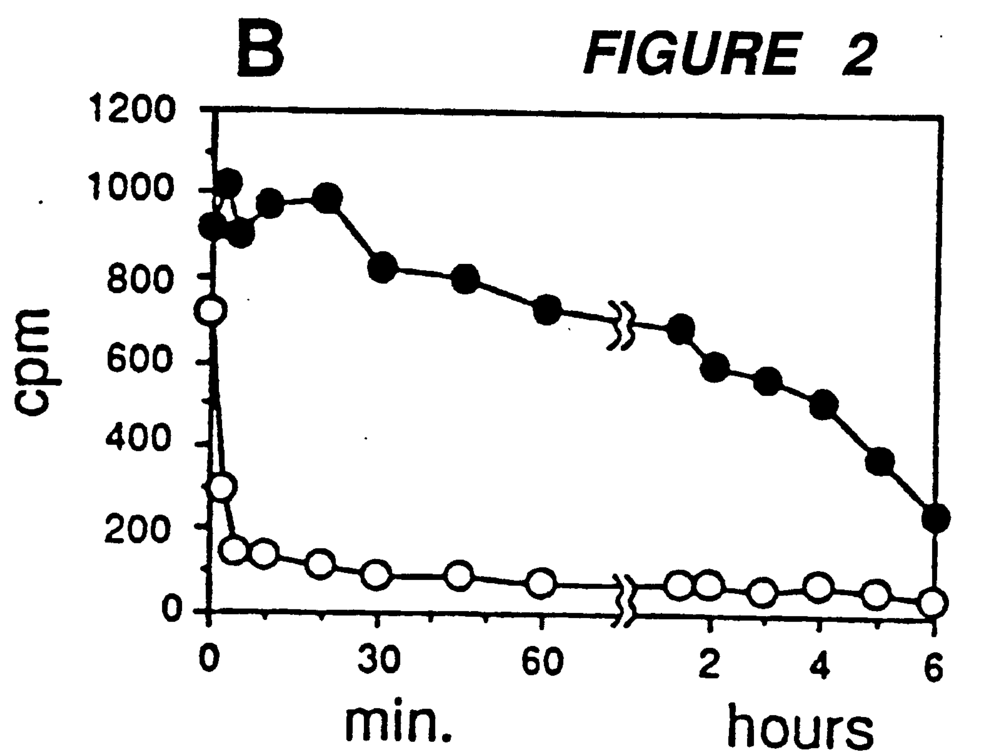

A
1200
1000
800
600
400
200
0
cpm
0
30
60
2
4
6
8
min.
hours

FIGURE 2


B
1200
1000
800
600
400
200
0
cpm
0
30
60
2
4
6
min.
hours

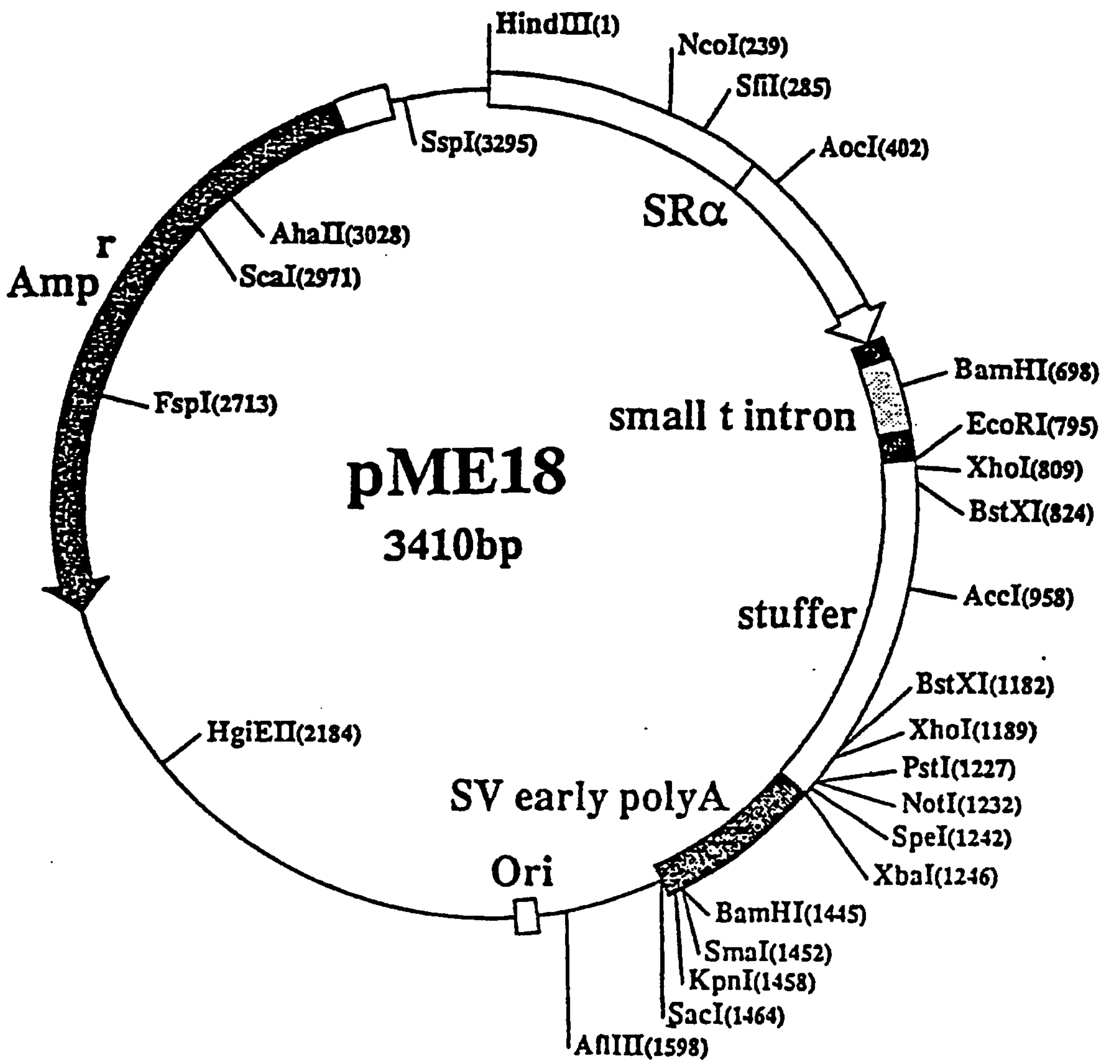

HindIII(1)
NcoI(239)
SfiI(285)
AocI(402)
SRα
BamHI(698)
small t intron
EcoRI(795)
XhoI(809)
BstXI(824)
AccI(958)
stuffer
BstXI(1182)
XhoI(1189)
PstI(1227)
NotI(1232)
SpeI(1242)
XbaI(1246)
SV early polyA
BamHI(1445)
SmaI(1452)
KpnI(1458)
SacI(1464)
AflIII(1598)
Ori
HgiEII(2184)
FspI(2713)
Amp r
ScaI(2971)
AhaII(3028)
SspI(3295)
pME18
3410bp

FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/04846

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; G01N33/50; G01N33/68; C07K13/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[°]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 87, December 1990, WASHINGTON US pages 9655 - 9659; Hyashida, K. et al.: 'Molecular cloning of a second subunit of the receptor for human granulocyte colony-stimulating factor (GM-CSF) : reconstitution of a high-affinity GM-CSF receptor.' see the whole document ---	1-13

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 DECEMBER 1991	20 DEC 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NAUCHE S.A.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, June 1991, WASHINGTON US pages 5082 - 5086; Kitamura, T. et al.: 'Reconstitution of functional receptors from human granulocyte/macrophage colony-stimulating factor (GM-CSF) : evidence that the protein encoded by the AIC2B cDNA is a subunit of the murine GM-CSF receptor.' see the whole document ---	1-13